European Journal of Pharmacology 744 (2014) 157–163

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural pharmacology

Pharmacological evaluation of sedative and hypnotic effects of schizandrin through the modification of pentobarbital-induced sleep behaviors in mice

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ARTICLE INFO

Article history:
Received 18 April 2014
Received in revised form
24 August 2014
Accepted 4 September 2014
Available online 19 October 2014

Keywords:
Schizandrin
Sedative–hypnotic effects
Pentobarbital sleep
Serotonergic system

Chemical compounds studied in this article:
Schizandrin (PubChem CID: 23915)

ABSTRACT

The fruits of *Schisandra chinensis* have been recorded as an effective somnificant for the treatment of insomnia in some oriental countries pharmacopoeias. However, the mechanism of sedative and hypnotic effects of this kind of herb is still unclear. In the present study, schizandrin, which is the main component of *Schisandra chinensis*, was selected as a target compound to investigate possible mechanisms through behavioral pharmacology methods. The results showed that schizandrin possessed dose-dependent (5–45 mg/kg, i.p.) sedative effects on locomotion activity in normal mice, and produced a dose-dependent decrease in sleep latency and an increase in sleep duration in pentobarbital-treated mice; thus, itself did not induce sleep at higher dose which was used in this experiment (45 mg/kg, i.p.). It also can reverse the rodent models of insomnia induced by *p*-chlorophenylalanine (PCPA) and caffeine, which could exhibit a synewith 5-hydroxytryptophan (5-HTP) as well; therefore, the hypnotic effects of schizandrin were not inhibited by flumazenil (a specific gamma aminobutyric acid (GABA)-A-BZD receptor antagonist). Altogether, these results indicated that schizandrin produces beneficial sedative and hypnotic bioactivity, which might be mediated by the modification of the serotonergic system.

1. Introduction

Insomnia is defined by the symptoms of difficulty falling asleep, repeated awakenings with difficulty returning to sleep, or sleep which is non-restorative or poor in quality. It is often accompanied by the perception of short overall sleep duration, which is widespread malady, with 10–15% of the adult populations suffering from chronic insomnia and an additional 25–35% with transient or occasional insomnia (Doghramji, 2006). The current treatments for insomnia involve sleep hygiene measures, behavioral therapies and pharmacological treatments (Roth and Drake, 2004; Zammit, 2007), and the last choice is the most common therapeutic method. However, hypnotics in clinical applications often show many untoward reactions, such as drug dependence, tolerance, rebound insomnia and other side effects. The search for novel herbal constituents from medicinal plants may provide better candidates for pharmacotherapeutics.

The fruit of *Schisandra chinensis* (Turcz.) Baill. (Schisandrae Fructus) has been used as a tonic for kidney and brain in traditional Chinese medicine for thousands of years, with the functions of inducing astringency, replenishing and promoting production of body fluid and tonifying the kidney to relieve mental strain. Therefore, *S. chinensis* may contain some promising neurotransmitter compounds having therapeutic usage for insomnia and convulsion. Although there are some reports about sedative and hypnotic activities of the ethanol fraction from *S. Chinensis* Fructus (Huang et al., 2007), scientific evidence and precise mechanism for their sedative–hypnotic activity have not been fully investigated.

Before the present study, we had authenticated that petroleum ether fraction from the 95% ethanol extract of *S. chinensis* exhibited significant sedative–hypnotic activity, and seven compounds were isolated from the PE extract. This study aimed to evaluate the effect of schizandrin from the active fraction and investigate the potential mechanism underlying its action. As one of the major bioactive constituents from the fruits of *S. chinensis*, the content range of schizandrin was from 28% to 50% in different batches (Lee and Kim, 2010; Liu et al., 2013), and schizandrin could pass through the blood–brain barrier and reach the brain (Wei et al., 2013), which is consistent with the traditional medicine. In this paper, after administering intraperitoneally with different doses of schizandrin, two kinds of behavioral pharmacology methods tests in mice were

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http://dx.doi.org/10.1016/j.ejphar.2014.09.012
0014-2999/

performed; furthermore, the mechanism and characters of sedative–hypnotic actions of schizandrin have also been explored on different models of insomnia mice. Finally, these results suggest that schizandrin produces beneficial sedative and hypnotic bioactivity, which might be mediated by the modification of the serotonergic system.

2. Materials and methods

2.1. Animals

Adult male Kunming mice (weighing 25 ± 2 g) were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). They were maintained under standard laboratory conditions of temperature 25 ± 1 °C and a 12 h light/12 h dark cycle with food and water available ad libitum for the duration of the study. After 1 week of acclimatization, all mice were randomly divided into different groups ($n=15$). The experimental procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication no. 85-23, revised 1985).

2.2. Drugs and materials

Schisandrae Fructus were collected from Liaoning province in China and identified by Professor Ying Jia (Department of Pharmacognosy, Shenyang Pharmaceutical University) according to the guid elines of the Chinese Pharmacopoeia (2010). Schizandrin (Wuweizichun A, schisandrol A, Wuweizisu) (Fig. 1, purity > 99%) was isolated by authors. Other drugs used in this study, flumazenil (FLU), *p*-chlorophenylalanine (PCPA), caffeine, and 5-hydroxytryptophan (5-HTP), were purchased from Melonepharma Co. Ltd. (Dalian, China), pentobarbital sodium was obtained from Merck Co. Ltd. (Shanghai, China), diazepam (DZP) injection was provided by Shenyang Hospital. All other chemicals and reagents were of the highest grade available.

2.3. Isolation and identification of schizandrin

Air-dried and powdered seeds of Schisandrae Fructus (3 kg) were exhaustively extracted with 95% aqueous EtOH (3×10 l) at reflux. The combined extracts were concentrated under reduced pressure to dryness. The residue was suspended in H_2O, and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The petroleum ether fraction was concentrated under reduced pressure to dryness. The residue (200 g) was subjected to column chromatography on silica gel and eluted with a gradient of petroleum ether–EtOAc (100% petroleum ether;100:1; 80:1; 60:1; 50:1; 30:1; 10:1; 5:1; 2:1; 100% EtOAc) to obtain 10 fractions. Fraction B (28 g) was further separated by silica gel column eluted with petroleum ether–acetone (100:1) to afford two subfractions, and Fraction B-2 was further separated on Sephadex LH-20 eluted with CH_3Cl–MeOH to afford three subfractions, Fraction B-2-2 (400 mg) was crystallized in MeOH to afford SC1 (350 mg), SC1 was isolated as a white crystalline compound, ESI–MS: *m/z* 433 $[M+H]^+$, 431 $[M-H]^-$; ^{1}H NMR(600 MHz, $CDCl_3$) δ: 0.82(3H, d, $J=7.0$ Hz, CH_3-8), 1.26(3H, s, CH_3-7), 1.88(H, m, H-8), 2.37(2H, m, H-6β, H-9α), 2.67(2H, m, H-6α, H-9β), 3.59(3H, s, OCH_3), 3.88(3H, s, OCH_3), 3.89(3H, s, OCH_3), 3.89(3H, s, OCH_3), 3.90(3H, s, OCH_3), 3.91 (3H, s, OCH_3), 6.54(H, s, H-11), 6.61(H, s, H-4). ^{13}C NMR(125 MHz, $CDCl_3$): 151.6(C-l), 140.2(C-2), 151.7(C-3), 110.6(C-4), 140.0(C-5), 39.5 (C-6), 33.9(C-7), 77.9(C-8), 35.7(C-9), 139.5(C-10), 107.1(C-11), 1530 (C-12), 139.9(C-13), 151.8(C-14), 122.5(C-15), 123.5(C-16), 12.7(C-17), 21.8(C-18), 55.8, 55.8, 60.5, 60.5, 60.9, 60.9 (6 × -OCH3). The data of 1H NMR and 13C NMR of SC1 was in accordance with literature report (Ikeya et al., 1979). Therefore, SC1's structure was identified as schizandrin.

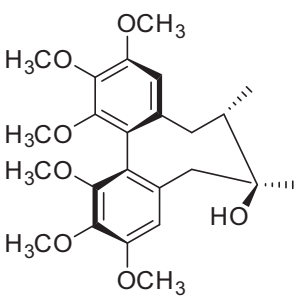

Fig. 1. Chemical structure of schizandrin used in this study.

2.4. Treatments

For intraperitoneal (i.p.) injection (0.05 ml/10 g), sodium pentobarbital was dissolved in physiological saline. The present study used 45 mg/kg (i.p.) as the hypnotic dose of sodium pentobarbital (with a 100% rate of sleep onset) and 30 mg/kg (i.p.) as the subhypnotic dose (with a 0% rate of sleep onset). Schizandrin (5, 15 and 45 mg/kg) and DZP (2 mg/kg) were dissolved with 1% dimethyl sulfoxide (DMSO) and administered i.p. 25 min before sodium pentobarbital administration (i.p.). In the agonism/antagonism experiments, 5-HTP was injected (i.p.) 15 min prior to the pentobarbital administration (i.p.), and FLU was administrated 15 min prior to the administration of schizandrin; models of PCPA-induced insomnia mice received an injection of PCPA (300 mg/kg, s.c.) between 08:00 and 09:00, 24 h prior to the administration of schizandrin. Models of caffeine-induced insomnia mice received an injection of caffeine (7.5 mg/kg, i.p.) between 13:00 and 13:30, 0.5 h prior to the administration of schizandrin. All drugs were prepared daily.

2.5. Behavioral analyses

Behavioral tests were performed in a soundproof room with a neutral environment. All of the tests were carried out between 08:30 and 11:30 or between 13:00 and 15:30, with matching between the groups. The observers were blind to the treatment.

2.5.1. Inner open-field behavior test

The sedative activity was investigated by determining the spontaneous locomotor activity of mice in an open field. Animals were placed in an open filed experimental video analysis system (ZS-ZFT, Huaibei Zhenghua Bio-apparatus Co. Ltd., China), and acclimated to the activity cages individually for 5 min. 25 min after injection with schizandrin (5, 15 and 45 mg/kg, i.p.), diazepam (2 mg/kg, i.p.) or 1% DMSO, the locomotion activity of each mouse was measured for 5 min. The interruptions of beams of two consecutive infrared sensors were collected for 5 min as a reflection of locomotor activity. After each testing session, the enclosures were thoroughly cleaned with 70% ethanol and water. The sedative property of schizandrin was evaluated using total path, time of immobility and numbers of active as indexes.

2.5.2. Pentobarbital-induced sleep test

Experiments were carried out between 08:00 a.m. and 11:30 a.m. or between 13:00 and 15:30. Following sodium pentobarbital injection, each mouse was observed for the onset of sleep. When the mice lost the righting reflex for over 1 min, they were considered to be asleep. The loss of righting reflex was defined as a failure of the mouse to right itself for at least 10 s after being placed on its back. Time elapsed between the administration of sodium pentobarbital

and sleep latency was recorded as the time of pentobarbital injection to the time of sleep onset, and sleep duration was defined as the elapsed time between the righting reflex loss and recovery. In the subhypnotic dosage of sodium pentobarbital treatment test, the percentage of sleep onset was calculated as follows: sleep onset (%)=no. falling asleep/total no. × 100.

2.6. Statistical analysis

All data are presented as the mean ± S.E.M. For statistical comparisons,the results were analyzed by a one-way analysis of variance (ANOVA) followed by the Students–Newman–Keuls test (SNK) for post-hoc comparisons. For the subhypnotic dosage of pentobarbital test, a chi-square test was used to compare the number of mice that fell asleep. Differences with $P<0.05$ were considered statistically significant.

3. Results

3.1. Sedative effect of schizandrin on the normal mice

The sedative activity of schizandrin was investigated by recording the spontaneous locomotor activity of mice. Compared with the vehicle group, the positive control DZP (2 mg/kg) induced a significant reduction in the percentage of locomotion length and increase in the percentage of immobility time ($P<0.01$); the number of counts was also reduced ($P<0.01$) (Fig. 2). Intraperitoneal administration of schizandrin (5, 15 and 45 mg/kg) also significantly decreased the percentage of locomotion length by 43%, 46%, and 47% and increased the percentage of immobi lity time by 59%, 49%,and 47%, respectively ($P<0.01$) (Table 1); the number of counts at the dose of 45 mg/kg was also reduced ($P<0.01$) (Fig. 1). Furthermore, except for schizandrin (SCH) 5 group, others also significantly decreased the number of counts ($P<0.05$) compared with themselves (Fig. 2).

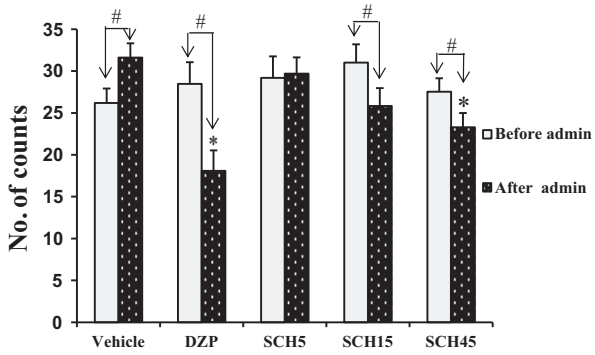

Fig. 2. Inhibitory effect of schizandrin on spontaneous locomotor activity in mice. Twenty five minutes after administration of DZP (2 mg/kg, i.p.) and schizandrin (5, 15 and 45 mg/kg, i.p.), spontaneous locomotor activity is measured. Each column represents the mean ± S.E.M, ($n=15$). $^{\#}P<0.05$, $^{*}P<0.01$ compared with the vehicle group.

3.2. Effect of schizandrin on the onset and duration of sleep in pentobarbital-treated normal mice

Schizandrin pretreatment significantly increased the rate of sleep onset with a subhypnotic dose of pentobarbital (30 mg/kg, i.p.) in a dose-dependent manner ($P<0.01$) compared with that of the vehicle group (Table 2). In mice treated with a hypnotic dose of pentobarbital (45 mg/kg, i.p.), schizandrin significantly potentiated the hypnotic effects of pentobarbital by reducing sleep latency at the dosages of 5 mg/kg ($P<0.05$), 15 mg/kg ($P<0.05$), and 45 mg/kg ($P<0.01$) (Fig. 3A) and prolonged sleep duration at the dosages of 5 mg/kg ($P<0.05$), 15 mg/kg ($P<0.05$), and 45 mg/kg ($P<0.001$) (Fig. 3B). In this experiment, we also found that schizandrin administered alone did not induce sleep.

3.3. Effect of schizandrin on the hypnotic-reversing action of flumazenil in pentobarbital-treated mice

Gamma aminobutyric acid (GABA)ergic neurotransmission plays a key role in sleep regulation, and the benzodiazepines (BZD) binding site on the GABA-A receptor is the target for most hypnotic and anxiolytic drugs (Bateson, 2006). The GABA-A-BZD antagonist FLU inhibits the sedative–hypnotic activity of GABA-A-BZD agonists, such as DZP, by preventing them from binding to GABA-A-BZD receptors (Johnston, 2005). In this research we found that to be true in our model system, FLU significantly ($P<0.001$) inhibited DZP hypnotic activity through increasing sleep latency ($P<0.001$, Fig. 4A) and decreased sleep time ($P<0.001$, Fig. 4B) compared with those of the normal mice. To verify the possible GABAergic mechanism of the hypnotic effects of schizandrin, the effects of schizandrin administered together with FLU were investigated, and the results authenticated that FLU showed no significant attenuation ($P>0.05$) in the potentiation of schizandrin on pentobarbital-induced sleep (Fig. 4).

Table 2
The effect of schizandrin on the sleep onset of mice treated with a subhypnotic dose of sodium pentobarbital. Twenty five minutes after administration of 2% DMSO, DZP, and schizandrin, sodium pentobarbital (30 mg/kg, i.p.) was given to mice. The number of mice falling asleep was recorded ($n=15$).

Group	Dosage (mg/kg, i.p.)	No. of falling asleep/total	Sleep onset (%)
Vehicle	–	0/15	0
DZP	2	15/15	100[a]
Schizandrin	5	6/15	40[a]
	15	10/15	67[a]
	45	14/15	93[a]

[a] $P<0.01$, compared with the vehicle group, chi-square test.

Table 1
Effect of schizandrin on locomotion activity in mice ($n=15$).

Group	Dosage (mg/kg, i.p.)	Locomotion length (cm/5 min)		Inhibitory (%)	Immobility time (s/5 min)		Increase (%)
		Before admin	After admin		Before admin	After admin	
Vehicle	–	752 ± 102	726 ± 144	0	84 ± 18	92 ± 24	0
DZP	2	979 ± 184	224 ± 62	71[a]	89 ± 27	155 ± 31	83[a]
Schizandrin	5	909 ± 176	617 ± 105	43[a]	113 ± 42	183 ± 35	59[a]
	15	866 ± 212	519 ± 166	46 [a]	102 ± 29	143 ± 35	49[a]
	45	796 ± 197	438 ± 142	47 [a]	126 ± 20	177 ± 40	47[a]

[a] $P<0.01$, compared with vehicle group.

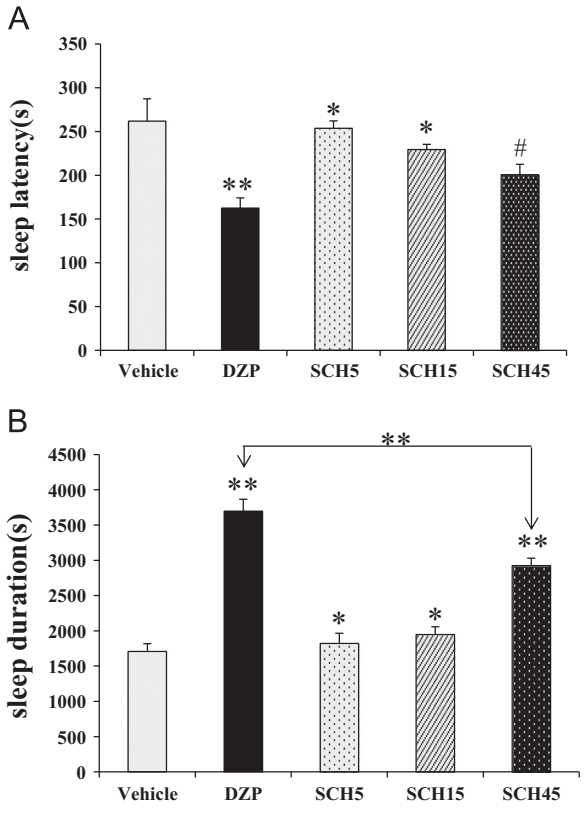

Fig. 3. Effect of schizandrin on the hypnotic response to pentobarbital-induced sleep in normal mice. The sleep latency (A) and the sleep duration (B) are assessed. All data are presented as mean ± S.E.M ($n=15$), $^{*}P<0.05$, $^{\#}P<0.01$, $^{**}P<0.001$.

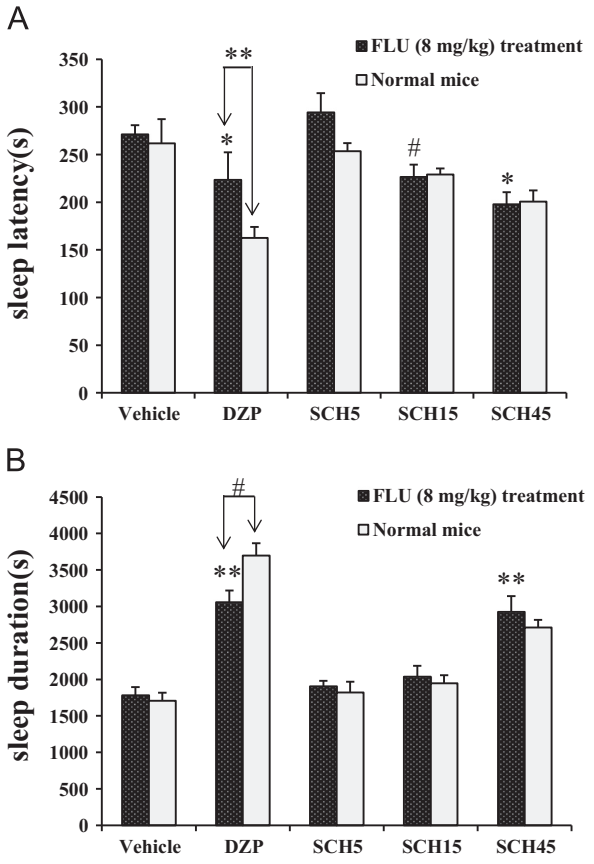

Fig. 4. Effects of the administration of schizandrin alone and with FLU on sleep latency and sleep duration in mice induced with pentobarbital (45 mg/kg, i.p.). The mice receive pentobarbital 25 min after administration of 2% DMSO, DZP, and schizandrin. FLU is injected 15 min before schizandrin administration intraperitoneally. All data are presented as mean ± S.E.M. $^{*}P<0.01$, $^{**}P<0.001$ vs. the vehicle group, $^{\#}P<0.05$ vs. the normal mice, no significant difference between the FLU treatment and non-FLU treatment except for DZP group is observed.

3.4. *Effect of schizandrin on PCPA-induced insomnia in pentobarbital-treated mice*

In accordance with the previous report (Borbely et al., 1981), the present study showed that treatment with PCPA (300 mg/kg, s.c.) 24 h prior to pentobarbital injection (45 mg/kg, i.p.) induced absolute insomnia in this study; however, treatment with schizandrin significantly attenuated the insomnia effect of PCPA in pentobarbital-treated mice, by increasing sleep latency ($P<0.001$, Fig. 5A) and decreasing sleep time ($P<0.001$, Fig. 5B) compared with that of the normal mice.

3.5. *Synergic effects of schizandrin and 5-HTP on sleep induced by pentobarbital*

To investigate the relationship between the hypnotic activity of schizandrin and the serotonergic system, the mice were treated with schizandrin (5 and 15 mg/kg) for 25 min and with 5-HTP (2.5 mg/kg) for 15 min prior to the administration of pentobarbital (45 mg/kg, i.p.). Neither schizandrin (5 mg/kg) nor 5-HTP (2.5 mg/kg) administered individually affected the sleep latency or sleeping time induced by the hypnotic dose of pentobarbital (Fig. 6A and B), and the rate of sleep onset induced by the subhypnotic dosages of pentobarbital was not influenced as well (Table 3). However, co-administration of schizandrin (5 and 15 mg/kg) and 5-HTP (2.5 mg/kg) exhibited the synergistic effect of shortening sleep latency ($P<0.05$, Fig. 6A) and prolonged sleeping time significantly ($P<0.05$, Fig. 6B). The co-administration significantly increased the rate of sleep onset ($P<0.05$, Table 3) in mice treated with subhypnotic dosages of pentobarbital as well.

3.6. *Effect of schizandrin on the caffeine-induced sleep disturbances in mice*

Rat treated with caffeine has been used as a model of insomnia in previous studies (Paterson et al., 2007). The present study showed that treatment with caffeine (7.5 mg/kg, i.p.) 0.5 h prior to pentobarbital injection significantly prolonged sleep latency ($P<0.001$, Fig. 7A) and decreased sleep time ($P<0.001$, Fig. 7B) compared with those of the normal mice. Schizandrin could significantly attenuate the insomnia effect of caffeine in pentobarbital-treated mice, by decreasing sleep latency ($P<0.001$, Fig. 7A) and increasing sleep time ($P<0.001$, Fig. 7B) compared with that of the vehicle group.

4. Discussion

Sleep and wakefulness are controlled by a network of brain nuclei which interacts in a complex fashion, integrating homeostatic and circadian regulations (Fuller et al., 2006; Siegel, 2004). Accordingly, insomnia is a sophisticated nervepathological state induced by the dysfunction of a network of neuronal systems. Because of easy accessibility and time-saving, pharmacological treatments often represent good options to gain access to, and maintain, good sleep in the current treatments for insomnia. Therefore, the potential efficacies of abundance of novel hypnotic drugs were determined during the pre-clinical phase of drug development in order to screen the best candidates and predict

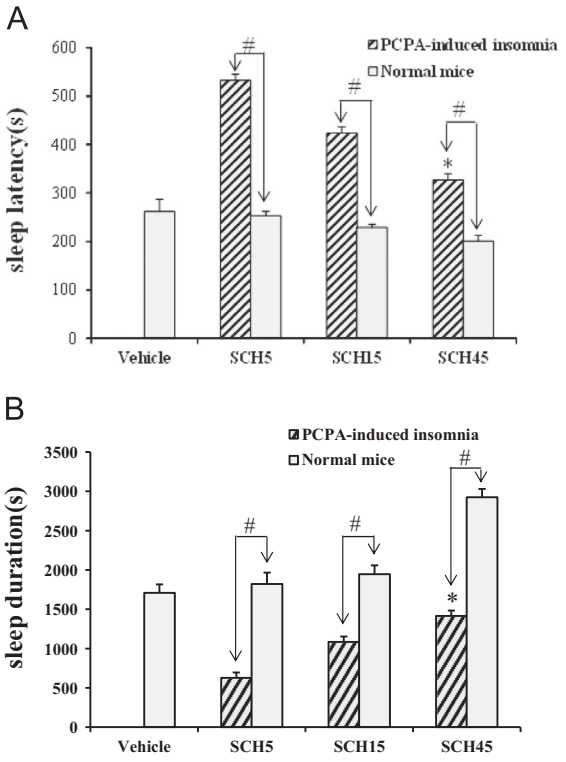

Fig. 5. Effects of schizandrin on PCPA-induced insomnia in pentobarbital-treated mice ($n=15$). Mice are pretreated with PCPA (300 mg/kg, s.c.) for 24 h and schizandrin for 25 min prior to the injection of pentobarbital (45 mg/kg). The sleep latency (A) and the sleep duration (B) are assessed. All data are presented as mean ± S.E.M. $^{*}P<0.01$ vs. vehicle group and $^{\#}P<0.001$ vs. normal mice.

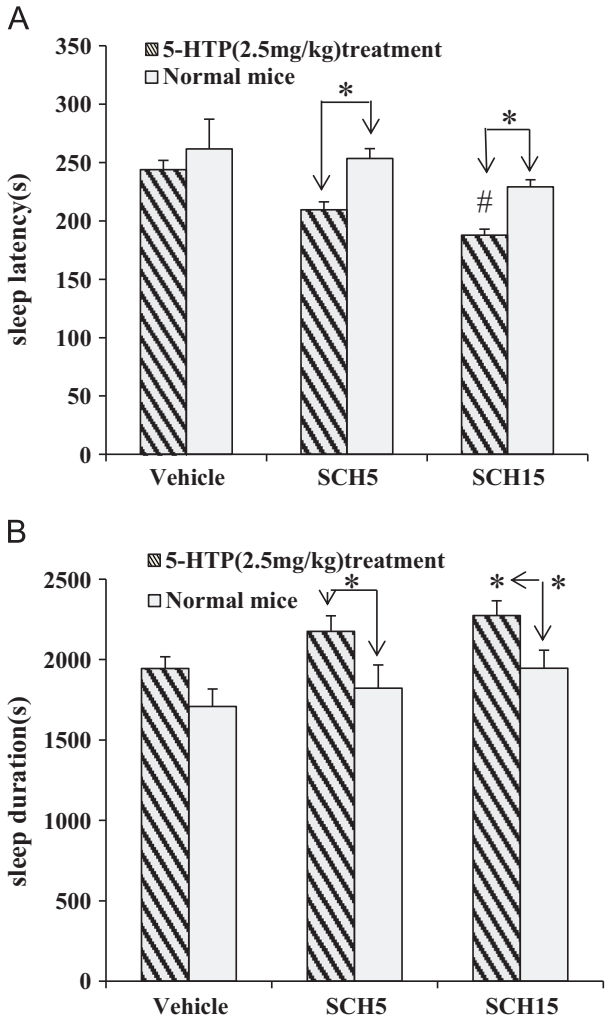

Fig. 6. Synergic effects of schizandrin with 5-HTP on hypnotic response in pentobarbital treated mice ($n=15$). Mice are administered schizandrin (5 and 15 mg/kg) for 10 min prior to and 5-HTP (2.5 mg/kg) for 15 min prior to the injection of pentobarbital (45 mg/kg). The sleep latency (A) and the sleep duration (B) are assessed. All data are presented as mean ± S.E.M., $^{*}P<0.05$ vs. normal mice, $^{\#}P<0.01$ vs. vehicle group.

the effects in humans. Currently, hypnotic drugs focus on the inh ibitory neurotransmitter GABA in the brain (Harrison, 2007; Lancel, 1999); however, the related hypnotic agents have many unpleasant side effects, including drug dependence, drug tolerance, and rebound insomnia. New treatments are emerging, and although the GABA receptor is still a target of choice, other biological targets are being considered. Active research is ongoing and a new generation of hypnotic drugs is expected to be developed, having efficacy to induce physiologic sleep together with enhanced safety and tolerability (Wafford and Ebert, 2008).

Schizandrin was originally isolated from *Schisandra chinensis*. Previous studies have focused on the neuronal protective effect of schizandrin both in vitro and in vivo (Hu et al., 2012). However, as one of the major bioactive constituents from traditional Chinese medicine for therapeutic use for insomnia, the central nervous system depressant effect of schizandrin has not been evaluated. Therefore, we characterized the sedative and hypnotic property of schizandrin. In order to investigate the activity and characters of sedative–hypnotic actions of schizandrin, the inner open-field behavior test and pentobarbital-induced sleep test in mice were performed, which are two classic behavioral pharmacology methods to evaluate the sedative–hypnotic activity (Zhu et al., 1996). Spontaneous motor activity is considered an index of alertness and a decrease in motor activity leads to sedation (Oztürk et al., 1996) as a result of the reduced excitability of the central nervous system (Masur et al., 1971). The sedative effects of drugs could be evaluated by measurement of pentobarbital-induced sleeping time in laboratory animals.

The present study showed that schizandrin significantly decreased locomotor activity (Table 1; Fig. 1) and potentiated the hypnotic effect of sodium pentobarbital (Table 2; Fig. 2) in normal mice, indicating its central nervous depressant activity, which is in accordance with the record of *S. chinensis* traditional therapeutic use for insomnia.

Table 3
Synergic effects of schizandrin with 5-HTP on sleep onset of mice treated with subhypnotic dosage of pentobarbital (30 mg/kg) ($n=15$).

Group	Dosage (mg/kg, i.p.)	No. of falling asleep/total	Sleep onset (%)
Vehicle	–	0/15	0
DZP	2	15/15	100[b]
5-HTP	2.5	3/15	20[a]
SCH	5	6/15	40[a]
5-HTP+SCH	2.5+5	15/15	100[b]

[a] $P<0.01$, compared with vehicle group, chi-square test.
[b] $P<0.001$ compared with vehicle group, chi-square test.

Moreover, the mechanism of sedative and hypnotic effects of schizandrin has also been investigated; in order to research whether the effects are based on positive allosteric modulation of GABA-A receptor or not, the experiment on effect of schizandrin on the hypnotic-reversing action of flumazenil in pentobarbital-treated mice was carried out. The result (Fig. 3) showed that schizandrin was not GABA-A-BZD receptor agonist, which implied that schizandrin induce sleep was not by positive allosteric modulation of the GABA-A-BZD receptor.

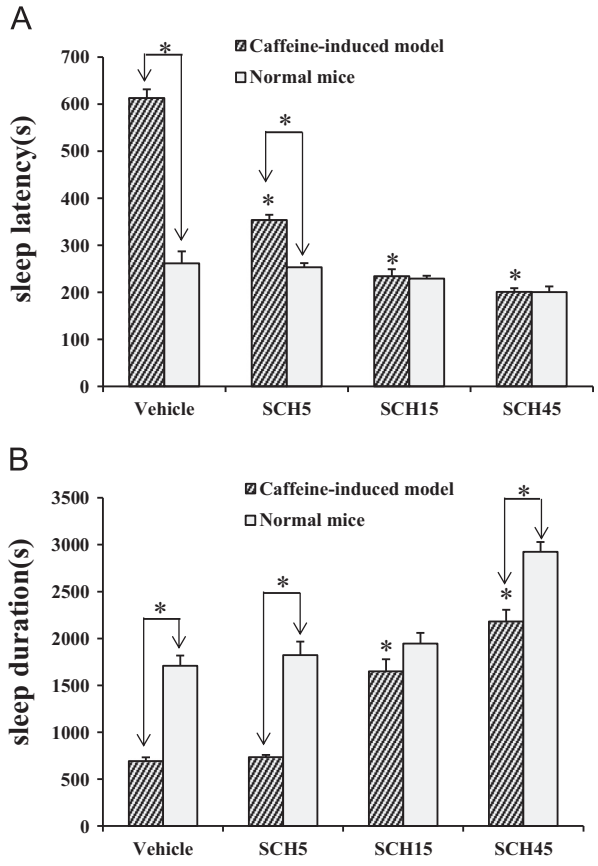

Fig. 7. Effects of schizandrin on caffeine-induced insomnia in pentobarbital treated mice ($n=15$). The sleep latency (A) and the sleep time (B) are assessed. All data were presented as mean ± S.E.M., ($n=15$), $^{*}P<0.001$.

Based on electrophysiological, neurochemical, genetic and neuropharmacological approaches, it is currently accepted that serotonin is not only associated with the initiation and maintenance of sleep but also plays a role in inhibiting sleep and promoting wakefulness. The complex effects of serotonin on the sleep–wake cycle are attributed to serotonin acting at different brain sites associated with the control of sleep and wakefulness. Previous studies have reported that different 5-HT receptors are selectively involved in the physiological functions (Dugovic, 2001; Zhang and Fang, 2001). The immediate serotonin precursor, 5-HTP, appears to have a general deactivating effect on the waking state (Ursin, 1976) and primarily induces drowsiness (Ursin, 2002), which also could prolongs pentobarbital-induced sleeping time in a dose-dependent manner (Zhao et al., 2004). In addition, complete insomnia or substantially reduced sleep was induced by chronic administration of PCPA, a serotonin (5-HT) synthesis inhibitor (Borbely et al., 1981). These insomnia effects were reversed by treatment with 5-HTP, which may have restored serotonin synthesis and thus restored sleep (Zhao et al., 2004). The serotonin–sleep connection, therefore, was proposed (Jouvet, 1984), in which the serotonin system was proposed to be hypnogenic. This PCPA/5-HTP model consolidates the importance of 5-HTP and 5-HT in the control of sleep (Smith and Kennedy, 2003). To investigate whether the hypnotic and sedative activities of schizandrin were related to the serotonergic system, the effect of 5-HTP/PCPA on the hypnotic activity of schizandrin was evaluated. The present study showed that schizandrin exerted synergic effects with 5-HTP on the rate of sleep onset with a subhypnotic dose of pentobarbital (Table 3) and on both sleep latency and sleep time with a hypnotic dose of pentobarbital in mice as well (Fig. 5). Moreover, schizandrin inhibited PCPA-induced suppression of the hypnotic effect of pentobarbital (Fig. 4). These results suggested that the serotonergic system may be involved in the potentiating mechanism of schizandrin's effects on the hypnotic effect of pentobarbital.

Sleep is a sophisticated physiological process generated by a network of neuronal systems that are hardly reproduced in-vitro, since there is probably no perfect model. Nevertheless, caffeine administration provides a simple and effective model of one of the most common symptoms of insomnia, difficulty in sleep initiation. Furthermore, as caffeine not only increases arousal, but also reduces the build-up of sleep pressure occurring during waking, it appears well-suited to reproduce the underlying feature of insomnia (Landolt, 2008; Paterson et al., 2007; Richardson, 2007). Finally, the caffeine model possesses additional advantages that make it an excellent insomnia model: it is simple and cost-effective, it is widely used and safe, and it can be used both in animals and humans. The present study also showed that schizandrin could partially counteract caffeine-induced sleep disturbances in mice (Fig. 6).

In summary, to our knowledge, this is the first study to explore the possible mechanism by which schizandrin augments the hypnotic effect of pentobarbital in different models of insomnia mice. This work demonstrates that the serotonergic system may be involved in the hypnotic–sedative activity of schizandrin. Further studies are needed to investigate the precise mechanism of this effect and elucidate the 5-HT receptor subtypes which may be involved in the potentiating effect of schizandrin on pentobarbital induced sleep.

Role of funding source

This research was supported by Shenyang Scientific Project (Nos. F13-287-1-00, and F12-153-9-00) and Liaoning Province Scientific Project (No. 2011412004).

Acknowledgments

Mr. Shaohuai Shi and Zhengzheng Liao, Ms. Bing Liu and Huan Li collaborated in the behavioral experiments. All the authors contributed and agreed to the final draft of the paper.

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